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Chemical Structure and Properties of Selected Benzene Compounds in Relation to Biological Activity

by V. H. Freed* and R. Haque*

Four fundamental physical parameters of series of benzene derivatives were studied to ascertain correlations with biological activity. These included octanol/water coefficient, vapor pressure, infrared absorption (out-of-plane C-H vibration), and the π values. Each physicochemical constant exhibited a partial correlation with biological activity suggesting that each played a role. It was presumed that more precise and specific data on the biological activity would have permitted a better correlation. It is postulated that the biological activity of a compound is due to the sum of such physicochemical factors as: partition coefficient, molecular geometry and spatial orientation, electronic characteristics of the molecular orbitals, and vibrational-rotational frequencies of constituent groups.

Introduction

The biological activity (toxicity) of benzene and its simple derivatives has long been recognized. Not only the parent hydrocarbon, but benzenes substituted with amino, nitro, halogen, and hydroxyl groups have been extensively studied (*1–3*). The biological effects of benzene and its simple derivatives may range from odor sensation to production of lesions in blood, liver, kidney, and nervous tissue (*1*). In addition to being toxic to mammals, many of these derivatives show marked biological activity on a wide variety of other organisms including bacteria, plants, insects, and fungi (*4*).

The type and intensity of the biological effect produced varies with the nature of the substituent on the benzene ring. Thus, the effects of phenolic derivatives differ from the nitro derivatives and from the halogen derivatives. Where multiple substitution occurs (either with the same or dissimilar substituents), the biological activity varies with the position of the substituent (*1, 3*). Thus, biological activity of 1,2- (*ortho*) disubstituted benzenes, may be markedly different from the 1,3- or 1,4-substituted benzene. In a study of odor intensity of chlorine-substituted phenols, marked differences were found in threshold concentrations, the 2,6-dichloro being much more pronounced in odor intensity than the 2,4-dichloro.

The type of substituents on benzene markedly influences the property of the compound as well as the biological effect. In study of the relationship of structure and physical properties to biological activity of the simple substituted benzenes, investigators have examined not only the positional isomerism, but also the physical properties such as vapor pressure, solubilities, steric and inductrometric effects of substituents (*5*) and attempted to correlate these to the biological activity. It is obvious that in any congeneric series of substituted benzenes, the biological activity is a summation of several molecular parameters, which may be expressed as in eq. (1):

$$A = \Sigma f(P, \sigma, E, p, S, \Delta H) \qquad (1)$$

where P, σ, E, p, S, ΔH are partition coefficient, Hammett constant, electronic parameter, vapor pressure, water solubility, and heat of solution, respectively.

*Environmental Health Sciences Center, Oregon State University, Corvallis, Oregon 97331.

One of the early attempts to deal with the complexity of the relationship of structure and physicochemical properties to biological activity of benzene derivatives was thermodynamic (*2*). In this instance, the biological activity was expressed in terms of ratio of vapor densities or solubilities:

$$\mu = p/p_0 \text{ or } S/S_0 \qquad (2)$$

where μ is activity, p_0 is saturated vapor density, S_0 is water solubility, p is vapor density at a predetermined activity level, and S is concentration of chemical.

However, examination of these problems suggests that the relationship is rather one of linear free energies contributed by several parameters; in other words, is extra thermodynamic (*6–8*). We must, therefore, take into account solubility characteristics, steric factors, and reactivity. Since in some instances biological effect expressed requires interaction of the whole molecule with some active site, the dimensions and spatial orientation of the molecule must also be considered (*9*):

$$\text{Toxicant} + \text{Enzyme} \rightleftharpoons \text{Complex} \qquad (3)$$

Spectroscopy, in certain regions of the electromagnetic spectrum, has been shown to be particularly useful for examining spatial orientation and to some extent dimensional relationships. Infrared spectroscopy is particularly useful for investigation of intramolecular orientation and energy relationships. It has been shown, for example, that the odor of certain substituted benzenes could be correlated with their infrared spectra (*4, 10–12*). Similarly, biological activity of certain other benzene derivatives were found to have a relationship to the infrared spectra in those regions that measured the spatial orientation of the substituents (*3*).

In this paper, a variety of physicochemical and structural properties of the series of substituted benzenes were examined for a possible relationship to biological activity. Among the factors investigated were the partition coefficients, π values, and infrared spectra.

Physicochemical Properties of Selected Aromatic Hydrocarbons

Data on such physicochemical properties of selected aromatic hydrocarbons as vapor pressure, octanol–water partition coefficient, and infrared vibration frequencies were collected. The partition coefficient values were used to calculate the constant π according to eq. (4):

$$\pi = P_x - P_H \qquad (4)$$

where P_x and P_H are the partition coefficients for the compound under consideration and the parent compound, respectively. In this case, the parent compound is benzene. The vapor pressure, P, and π values are given in Table 1. The lethal dose LD_{50} values for these chemicals are also given in Table 1.

Table 1. Physicochemical properties of aromatic hydrocarbons.

	Log LD_{50}[a]	log P[b]	π	Vapor pressure p[c]	Log p
Benzene	3.611	2.13	0.000	75.10	1.88
Aniline	2.645	0.90	−1.23	0.49	−0.31
Nitrobenzene	3.813	1.85	−0.28	0.255	−0.59
Phenol	2.724	1.46	−0.67	0.211	−0.68
Chlorobenzene	3.464	2.84	0.71	8.76	0.94
Toluene	3.806	2.73	0.56	22.4	1.35
3-Chloroaniline	2.944	1.88	−0.25		
4-Chloroaniline	2.477	1.83	−0.32	0.0155	−1.81
3,4-Dichloroaniline	2.845	2.69	0.56		
1-Chloro-3-nitrobenzene	3.391	2.46	0.33		
1-Chloro-4-nitrobenzene	2.623	2.39	0.26	0.0128	1.89
4-Nitroaniline	3.301	1.39	−0.74		
1-Hydroxy-2-methylbenzene	3.130	1.95	−0.18	0.168	−0.77
1-Hydroxy-3-methylbenzene	3.305	1.96	−0.17	0.092	−1.04
1-Hydroxy-4-methylbenzene	3.256	1.94	−0.19	0.067	−1.17
N,N-Dimethylaniline	3.130	2.31	0.18	0.43	−0.37

[a] From *Handbook of Toxicology* (*13*).
[b] Data of Leo et al. (*14*).
Data of Timmermans (*15*).

The aromatic hydrocarbons chosen in this study include benzene, aniline, nitrobenzene, phenol, chlorobenzene, toluene, 3-chloroaniline, 4-chloroaniline, 3,4-dichloroaniline, 1-chloro-3-nitrobenzene, 1-chloro-4-nitrobenzene, 4-nitroaniline, 1-hydroxy-2-methylbenzene, 1-hydroxy-3-methylbenzene, 1-hydroxy-4-methylbenzene, and *N, N*-dimethylaniline.

Discussion

The correlation of physicochemical properties of aromatic hydrocarbons with toxicity data is rather a difficult problem. The two main problems are the unavailability of consistent and reliable LD_{50} data and the fact that aromatic hydrocarbons represent a group of large numbers of compounds possessing different types of substituents at the ring. However, we may correlate the LD_{50} values with some of the physicochemical properties.

Many aromatic hydrocarbons have an effect on the central nervous system. If one assumes that the transport of the chemical in the lipid and membranes is one of the principal factors associated with the toxicity, then we should have some correlation between LD_{50} and the lipid/water partition coefficient of these chemicals P, according to eq. (5):

$$\log LD_{50} = A \log P + B \qquad (5)$$

In eq. (5) A and B are constants. A plot of log LD_{50} versus log P is shown in Figure 1. A linear regression analysis produced constants A and B as 2.149 and −1.24, respectively. The significance of the correlation is moderate to low since the correlation coefficient was only 0.36. The low correlation is not unrealistic in view of the two main points discussed above. However, we may say in general that as the partition coefficient increased the LD_{50} also shows an increase. This also suggests that the transport of aromatic hydrocarbons in lipids and membranes is of some importance.

While studying the structure–activity relationship for drugs, Hansch (*4*) proposed the correlation equation (6):

$$\log LD_{50} = C\pi^2 + D\pi + E\sigma + F \qquad (6)$$

where π is defined as earlier, σ is the Hammett constant, and C, D, E, and F are constants. Attempts were made to test such a correlation. In view of the large number of compounds in the list, it is difficult to come up with σ values. Therefore, a test was made for eq. (7):

$$\log LD_{50} = C\pi^2 + D\pi + F \qquad (7)$$

Such a plot is shown in Figure 2. The constants C, D, and F were found to be −0.128, 0.247, and 3.209, respectively. Again, the correlation coefficient was only 0.41, indicating only a partial correlation.

Attempts were also made to find a correlation between vapor pressure and LD_{50} (Fig. 3). It is evident from the scatter in the figure that there is a limited correlation between vapor pressure and the LD_{50}

Several workers have attempted in the past to find a correlation between infrared vibration frequency and biological activity. Wright (*8,11,12*) found a relationship between infrared vibration

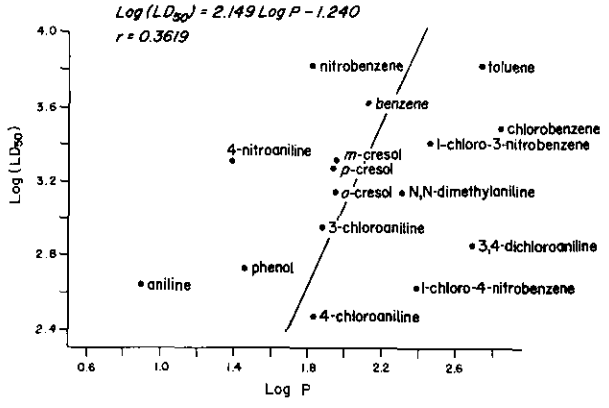

FIGURE 1. Relationship between partition coefficient and LD_{50} of aromatic hydrocarbons.

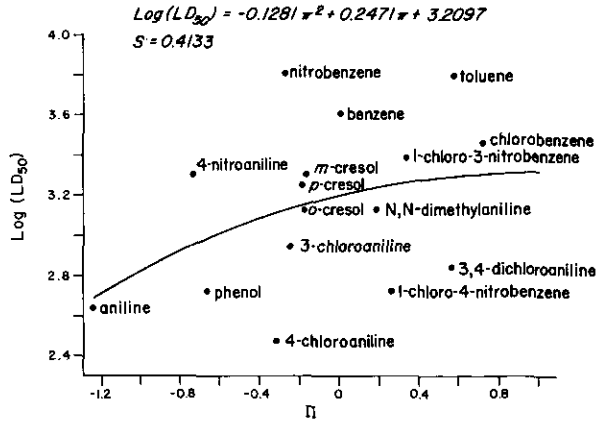

FIGURE 2. Relation of π and log LD_{50} according to $\log LD_{50} = 0.128\pi^2 + 0.2471\pi + 3.2097$.

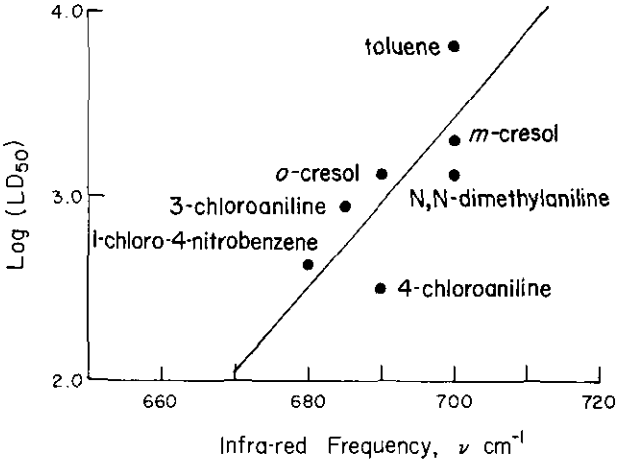

FIGURE 3. Vapor pressure and LD_{50} relationship.

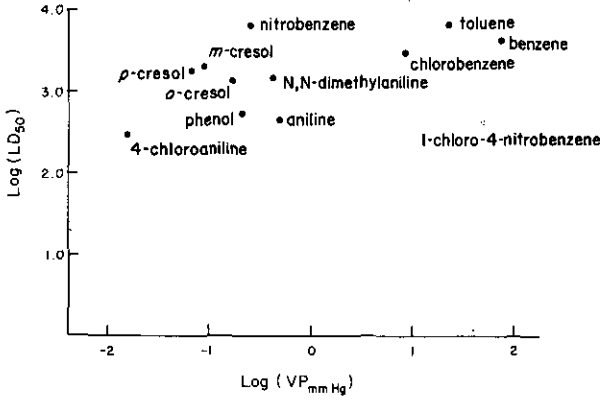

FIGURE 4. Graph showing LD_{50} and out-of-plane C-H vibration of aromatic hydrocarbons.

frequencies and odor of organic molecules. While studying the mode of action of organophosphate insecticides, Fukuto and Metcalf (*16*) found a linear correlation between phosphorus–oxygen–aromatic stretching frequency and fly-brain cholinesterase I_{50}. In the present study the possible correlation of toxicity to infrared spectra was investigated to find a similar correlation. In view of the complex nature of the infrared spectra of most of the compounds one must look for specific peaks to correlate with biological activity. One such correlation between LD_{50} and an intense peak in the region 640–740 cm^{-1} was identified. This intense peak corresponds to the C-H out-of-plane vibration of the benzene ring (Fig. 4). It is interesting to note that there is some correlation also between LD_{50} and the ring out-of-plane vibration. This suggests that the out-of-plane vibration of the ring may be important in the mode of action of these chemicals.

From the above examination it may be said that the partition coefficient, geometry, electronic factors, and the vibrational energies of aromatic hydrocarbons are important in the mode of action and intensity of toxicity. They may thus serve a basis of prediction and evaluation of threshold concentration levels.

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REFERENCES

1. Albert, A Selective Toxicity, Methuen, London, 1968.
2. Ferguson, J. Relations between thermodynamic indices of narcotic potency and the molecular structure of Narcotics. In: Centre National de la Recherche Scientifique, Colloques Internationaux 26, CNRS, Paris, 1951.
3. Sasaki, Y., and Suzuki, M. Studies on the biological activity. II. Discussions on the biological activity of *para*- and *meta*-substituted benzene derivatives. Chem. Pharm. Bull. (Tokyo) 17: 1569 (1969).
4. Hansch, C. A quantitative approach to biochemical structure–activity relationships. Accts. Chem. Res. 2: 232 (1969).
5. Fukui, K., Nagata, C., and Yonezawa, T. Electronic structure and auxin activity of benzoic acid derivatives. J. Amer. Chem. Soc. 80: 2267 (1958).
6. Pianka, M., and Edwards, J. D. Structures and pesticidal activities of derivatives of dinitrophenols. VIII.—Effects of substitution with C_5 to C_{13}-s-alkyl groups and of esterification on the acaricidal activity of dinitrophenols. J. Sci. Food Agr. 19: 60 (1968).
7. Sadler, P. W. Applications of molecular spectroscopy to relationships between biological activity and structure. Chem. Revs. 60: 575 (1961).
8. Wright, R. H. Odour and molecular vibration. I. Quantum and thermodynamic considerations. J. Appl. Chem. 4: 611 (1954).
9. Mullins, L. J. Some physical mechanisms in narcosis. Chem. Revs. 54: 289 (1954).
10. Sexton, H. W. Chemical Constitution and Biological Activity, 3rd ed., Van Nostrand, New York, 1963.
11. Wright, R. H. Odour and molecular vibrations. Nature, 190: 1101 (1961).
12. Wright, R. H., and R. S. Serenius. Odour and molecular vibration. II. Raman spectra of substances with the nitrobenzene odour. J. Appl. Chem. 4: 615 (1954).
13. National Academy of Sciences and National Research Council, Handbook of Toxicology, Saunders, Philadelphia.
14. Leo, A., Hansch, C., and Elkins, D. Chem. Revs. 71: 525 (1971).
15. Timmermans, J. Physicochemical Constants of Pure Organic Compounds, Elsevier, Amsterdam, 1950.
16. Fukuto, T. R. and Metcalf, R. L. Structure and insecticidal activity of some diethyl substituted phenyl phosphates. J. Agr. Food Chem. 4: 930 (1956).